

COMBINED PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS IDENTIFIES DIFFERENTIALLY EXPRESSED PATHWAYS ASSOCIATED TO *PINUS RADIATA* NEEDLE MATURATION

**Luis Valledor^{1,2}, Jose Luis Rodríguez^{1,2}, Christof Lenz³,
Roberto Rodríguez^{1,2}, Maria J. Cañal^{1,2} and Jesús Jorrín⁴**

¹EPIPHYSAGE Research Group, Área de Fisiología Vegetal, Departamento B.O.S.,
Universidad de Oviedo, Oviedo, Spain;

²Instituto Universitario de Biotecnología de Asturias (IUBA), Oviedo, Spain;

³Applied Biosystems Deutschland, Frankfurter Strasse 129-B, Darmstadt, Germany;

⁴Plant Proteomics-Agricultural and Plant Biochemistry Research Group,
Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, Córdoba, Spain.

Needle differentiation is a very complex process which leads to the formation of a mature photosynthetic organ from pluripotent needle primordia. We characterized and compared the proteome and transcriptome of immature needles (1 month old) and fully developed needles (12 months old) of *Pinus radiata* D. Don to characterize metabolic pathways implied in this process. After differential 2-DE (pH 5-8, 18 cm, CBB staining) 884 spots were analyzed defining 280 as differential (T-Test, Bonferroni correction for $\alpha=0.05$). Out these 280 spots, 134 were confidently identified by LC-ESI-Q-TRAP-MS employing a custom viridiplantae protein database (Applied Biosystems) and Paragon algorithm present in ProteinPilot Software (Applied Biosystems). Transcriptomic analyses were performed in three stages: 1. Two suppressive subtractive hybridization (SSH) libraries enriched with differential cDNAs were constructed for immature and mature needles. Libraries were constituted by 576 clones each, with 198 and 144 different sequences for immature and mature scions, respectively. 2. The differential expression of subtracted cDNAs was tested by hybridization over custom macroarrays (13 x 9 cm, 384 probes). 3. The expression level of 15 genes was determined by real time RT-PCR to validate macroarray results. A joint data analysis of proteomic and transcriptomic results was also performed to have a combined perspective which gives us a broad view over differentially expressed pathways associated to needle maturation. Energy metabolism pathways, with photosynthetic and oxidative phosphorylation related proteins, were overexpressed in mature needles. Aminoacid metabolism, transcription and translation pathways were overexpressed in immature needles. Interestingly stress related proteins and defense mechanisms were characteristic of immature tissues, and may be linked to the higher growth rate and capacity of response of this tissue.